bio-protocol Preprint

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Sucrose gradient fractionation

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An abbreviated version of this protocol was published in Science Advances in Dec 2019

Fidelity of translation initiation is required for coordinated respiratory complex assembly

DOI: 10.1126/sciadv.aay2118

Detailed protocol

1 mg of heart mitochondria, were lysed in 1% n-dodecyl β-D-maltoside (Sigma). Lysates were loaded on 10–30% sucrose gradients and separated by centrifugation overnight at 71, 000 g at 4˚C. Gradient fractions were collected as 750 µl aliquots. RNA was extracted from one third of each fraction by using the TRIzol LS Reagent (Invitrogen), according to the manufacturer's recommendations. The samples were subsequently treated with DNase I and used for cDNA synthesis (Qiagen). The transcript abundance in each fraction was assessed by qRT-PCR. The remainder of each fraction (500 µl) was precipitated with 72% trichloracetic acid, resolved by SDS- PAGE and OXPHOS or ribosome-containing fractions were detected by immunoblotting using antibodies specific for individual proteins.
For details on how to pour a sucrose gradient please follow the link below, it is all the same except we use 30% instead of 50% sucrose:
https://drummondlab.org/protocols/protocol/sucrose-gradient

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Filipovska, A. (2021). Sucrose gradient fractionation. Bio-protocol Preprint. bio-protocol.org/prep973.
2. Rudler, D. L., Hughes, L. A., Perks, K. L., Richman, T. R., Kuznetsova, I., Ermer, J. A., Abudulai, L. N., Shearwood, A. J., Viola, H. M., Hool, L. C., Siira, S. J., Rackham, O., Filipovska, A. and Filipovska, A.(2019). Fidelity of translation initiation is required for coordinated respiratory complex assembly . Science Advances 5(12). DOI: 10.1126/sciadv.aay2118